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Tumorigenesis and Neoplastic Progression

A Western-Type Diet Accelerates Tumor Progression in an Autochthonous Mouse Model of Prostate Cancer

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Epidemiological studies have provided evidence suggesting an important role for diet and obesity in the development of cancer. Specifically, lipid nutrients of the diet have been identified as important regulators of tumor development and progression. In the present study, we have examined the role of dietary fat and cholesterol in the initiation and progression of prostate cancer using the well-characterized TRAMP mouse model. Consumption of a Western-type diet—that is, enriched in both fat and cholesterol—accelerated prostate tumor incidence and tumor burden compared to mice fed a control chow diet. Furthermore, we also show that this diet increased the extent and the histological grade of prostate tumors. These findings were confirmed by the presence of increased levels of protein markers of advanced tumors in prostates obtained from animals fed a Western-type diet compared to those obtained from control animals. Increased lung metastases in animals fed a Western-type diet were also observed. In addition, we found that with a Western diet, animals bearing tumors presented with reduced plasma cholesterol levels compared with animals fed a control diet. Finally, we show that tumors obtained from animals fed a Western-type diet displayed increased expression of the high-density lipoprotein receptor SR-BI and increased angiogenesis. Taken together, our data suggest that dietary fat and cholesterol play an important role in the development of prostate cancer. ***(Am J Pathol 2010, 177:3180–3191; DOI: 10.2353/ajpath.2010.100568)***

According to cancer statistics published by the American Cancer Society, prostate cancer is the most frequently diagnosed cancer and the second-leading cause of cancer deaths among men in the United States (American Cancer Society, Cancer Facts & Figures 2010, *http://www.cancer.org/Research/cancer-facts-and-figures-2010*, last accessed October 15, 2010). There is strong evidence suggesting that genetic changes in epithelial cells of the prostate are almost inevitable with aging. Therefore, the prevalence of small, latent prostatic carcinomas is believed to be similar across many populations.[1] However, marked geographic variation exists in the clinical incidence of prostate cancer, ranging from 2.3 per 100,000 men in China to 101 and 137 per 100,000 men in white and black Americans.[2] Interestingly, the incidence of prostate cancer in Chinese and Japanese men increases substantially after migration to the United States.[3,4] Such differences may be attributable to the acquisition of more "Westernized" behaviors, such as change in diet, activity level, and/or use of substances such as tobacco and alcohol. Regarding the diet, migration of Asians to Western countries implies not only the consumption of more animal-based diets, but also a reduction in the consumption of a diet enriched in certain types of vegetables. In this case, consumption of phytochemicals has been demonstrated to potentially reduce cancer risk. These diets are enriched in polyphenols (found in green tea) and phytoestrogens (present in large quantities in soy products).[5] From these observations

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stems the hypothesis that environmental factors, most likely present in the diet, may act as late stage promoters responsible for the transformation of the prostate tumor from a latent form into a more aggressive and clinically-apparent form.[4]

Diet and obesity are now considered important risk factors for cancer development.[6,7] Experimental and epidemiological evidences have suggested that increased dietary fat intake may be associated with increased prostate cancer risk.[8–11] However, as the literature on the topic has expanded, the fat–cancer association has become more tenuous, with recent important studies not confirming this association.[12] Conversely, the specific dietary nutrients that may predispose to prostate cancer are not clear.[13] Several studies have demonstrated that cholesterol, a prominent lipid component of the Western diet, accumulates in solid tumors. In addition, cholesterol homeostasis is altered in the prostate with aging and during the transition to malignant tumors.[14–19] Nevertheless, the investigation of a possible relationship between dietary and plasma cholesterol levels and tumor incidence has offered contradictory results. In some epidemiological studies, a significant positive correlation has been obtained between hypercholesterolemia and prostate cancer incidence.[20] Additional research has demonstrated statistically significant correlations between dietary cholesterol intake and cancer risk.[21–23] Tumor progression may also be correlated with a progressive decrease in plasma cholesterol levels.[24] In this case, it has been suggested that decreased plasma cholesterol levels may be a metabolic consequence of the tumor existence instead of a cause. In agreement with these hypotheses, recent studies have even suggested that statin treatment of hypercholesterolemia may be associated with reduced prostate cancer incidence.[25] Taken together, these data suggest that increased plasma cholesterol levels may be a predisposing factor for cancer development. However, in later stages of cancer development, plasma cholesterol levels are reduced possibly because of an increased utilization by the developing tumors.[26]

In the present study, we have tested the hypothesis that increased dietary fat and cholesterol, and consequently, increased plasma cholesterol levels, may play an important role in prostate cancer onset and progression as well as in metastasis development. To evaluate this possibility, we have used the *t*ransgenic *a*denocarcinoma of the *m*ouse *p*rostate (TRAMP) model, which spontaneously develops prostate tumors. TRAMP male mice carry a transgene that allows the expression of the SV40 large T antigen under the control of a prostate-specific promoter (probasin). As a consequence, male TRAMP mice develop spontaneous multistage prostate cancer that exhibits both histological and molecular features similar to those observed in human prostate cancer cases. In this model, prostate cancer progresses from prostatic intraepithelial neoplasia (PIN) that is thought to be a precursor lesion, to adenocarcinoma. PIN is believed to lead to microinvasion, which subsequently results in frank invasion and metastatic disease.[27,28] Distant site metastases can be detected in male TRAMP mice as early as 12 weeks of age, and by 28 weeks of age a significant proportion of animals harbor prostate tumors that metastasize to the lymph nodes and lungs.[29,30]

Materials and Methods

Materials

Antibodies and their sources were as follows: rabbit polyclonal anti-cyclin D1 was from NeoMarkers (Fremont, CA); mouse monoclonal anti-PCNA was from Santa Cruz Biotechnology, Inc. (Palo Alto, CA); rabbit polyclonal anti-SR-BI was from Novus Biologicals, Inc. (Littleton, CO); and rabbit anti-CD31 was from Abcam, Inc. (Cambridge, MA).

Animal Studies

All animals were housed and maintained in a barrier facility at the Kimmel Cancer Center at Thomas Jefferson University. Mice were kept on a 12-hour light/dark cycle with *ad libitum* access to food and water. Animal protocols used in this study were approved by the Institutional Animal Care and Use Committee from Thomas Jefferson University. TRAMP (*t*ransgenic *a*denocarcinoma of *m*ouse *p*rostate) mice expressing the SV40 large T-antigen under the control of the prostate specific rat probasin promoter were obtained from The Jackson Laboratory (Bar Harbor, ME). All mice used in this study were in the C57Bl/6J background. Transgenic males were distinguished from their non-transgenic littermates for the presence of the TRAMP transgene by PCR, as suggested by The Jackson Laboratory (Bar Harbor, ME). All TRAMP mice used in this study were hemizygous for the TRAMP transgene. For tumor studies, 8-week-old TRAMP males and their nontransgenic littermates were distributed into either a chow diet (regular chow diet containing 4.5% fat and 0.002% cholesterol (wt/wt), TestDiet) or a Western diet (typical Western-type diet containing 21.2% fat and 0.2% cholesterol (wt/wt), TestDiet) and sacrificed at 28 weeks of age. Carbohydrate content (50% and 48% for chow and Western diet, respectively) and energetic values (4.14 and 4.43 kcal/g for chow and Western diet, respectively) were similar between the two diets. The number of mice for each experimental group ranged from 17 to 19.

Tissue Excision and Processing

At the time of sacrifice, total body weight and epididymal fat weight were determined for each mouse. The genitourinary (GU) tract including the bladder, seminal vesicles, ampulary gland, and prostate was excised *en bloc* and weighed. Whenever possible, the GU tract was further dissected under a dissecting microscope to excise the prostate individual lobes. Therefore, each pair of ventral, lateral, dorsal, and anterior lobes was microdissected and frozen in liquid nitrogen or fixed in 10% neutral buffered formalin. Whenever a gross tumor obscured the boundaries of the individual prostatic lobes and so that no individual isolation was possible, the whole

tumor mass was dissected, weighed, and formalin-fixed for further histopathological analysis.

Histopathological Analysis of Ventral and Anterior Prostate

Ventral and anterior prostate lobes dissected from male mice at 28 weeks of age were fixed with 10% neutral buffered formalin for 24 hours, transferred to 70% ethanol, dehydrated, and embedded in paraffin. Sections were cut at 5 μm, stained with hematoxylin and eosin, and evaluated by an experienced histopathologist without the knowledge of the mouse genotype or experimental group. Each prostatic lobe was graded as normal, prostatic intraepithelial neoplasia (PIN), well-differentiated adenocarcinoma (WD), moderately-differentiated adenocarcinoma (MD), and poorly-differentiated adenocarcinoma (PD) using a scale that had been previously established for TRAMP mice.[28,30] Sections from 8 to 10 mice were analyzed for each experimental condition. Computer-assisted image analysis was performed using an Olympus BX51 System Microscope (Olympus Corp., Miami, FL) equipped with a Micropublisher 5.0 cooled CCD camera (QImaging Corp., Burnaby, BC).

Lung Metastasis Analysis

After removal of the GU tract, 28-week-old TRAMP male mice were injected with 2 ml of 10% neutral buffered formalin by tracheal cannulation to fix the inner spaces and inflate the lung lobes. Lungs were then excised and placed into formalin for 24 hours. The left lung of each animal was paraffin-embedded, sectioned at 50-μm intervals, and stained with hematoxylin and eosin. Lung metastasis were scored as the total number of metastatic foci (defined as a cluster of 10 or more cells) per lung, as we previously described.[31] We analyzed the lungs obtained from 7 and 9 mice fed a chow or a Western diet, respectively.

Immunohistochemistry

Paraffin-embedded ventral prostatic lobes were first deparaffinized by treatment with xylene and then rehydrated by passage through a graded series of ethanol. Antigen retrieval was performed by placing the slides in a sodium citrate buffer solution in a pressure cooker. Endogenous peroxidase activity was quenched by incubation in 3% H_2O_2. Slides were then washed with PBS, blocked with 10% normal goat serum (Vector Laboratories, Inc., Burlingame, CA) in PBS for 1 hour, and incubated with the primary antibody diluted in blocking solution overnight at 4°C. Sections were then incubated with a biotin-streptavidin detection system (LSAB2 System-HRP, Dako North America, Inc., Carpinteria, CA), and bound antibodies were visualized using 3,3′–diaminobenzidine (DAB) as a substrate. Finally, slides were washed in PBS, counterstained with hematoxylin, dehydrated, and mounted with coverslips. Sections from four TRAMP males fed either a chow or a Western-type diet were stained. Computer-assisted image analysis was performed using an Olympus BX51 System Microscope (Olympus Corp., Miami, FL) equipped with a Micropublisher 5.0-cooled CCD camera (QImaging Corp., Burnaby, BC).

Plasma Cholesterol Determination

Plasma samples from TRAMP male mice and their nontrangenic littermates were obtained by tail bleeding at 8 (basal levels before distribution of the mice into the different diets) and 22 weeks of age, and by cardiac puncture after the mice had been sacrificed at 28 weeks of age. Total plasma cholesterol levels were determined using a colorimetric assay kit (Wako Chemicals USA, Inc., Richmond, VA). The number of mice in each experimental group ranged from 17 to 19.

Lipoprotein Profiles

Lipoprotein profiles were determined by fast protein liquid chromatography (FPLC). Fasting plasma samples obtained from 10 mice in each group were pooled to obtain a total volume of 150 μl and loaded onto a Superose 6 column (GE Health care Bio-Sciences Corp., Piscataway, NJ) to achieve a total bed volume of 25 ml and a void volume of 7.5 ml. Plasma was passed through the column at a flow rate of 0.25 ml/min, and 0.5-ml fractions were collected. Total cholesterol content of each fraction was determined (Wako Chemicals USA, Inc., Richmond, VA) and plotted against elution volume.

Statistical Analysis

Values were reported as the mean ± SE. Comparisons between control and treated mouse samples were performed using the Student's *t*-test or by analysis of variance when appropriate. The number of mice used for each experiment was indicated in the corresponding figure legend.

Results

A Western-Type Diet Accelerates Prostate Tumorigenesis

To directly assess the role of dietary fat and cholesterol on prostate tumor onset and progression, we fed 8-week-old TRAMP male mice with a chow diet (containing 4.5% fat and 0.002% cholesterol) or a Western diet (containing 21.2% fat and 0.2% cholesterol). On necropsy at 28 weeks, 33% (6 of 18 mice) of TRAMP mice fed a Western diet showed a grossly evident spherical prostate tumor. By contrast, the incidence of grossly-identifiable tumors in TRAMP mice fed a chow diet was only 17% (3 of 17 mice) (Figure 1A). Whenever present, prostate bulky tumors were carefully excised and weighed. Taking the total tumor weight per mouse into account, TRAMP mice

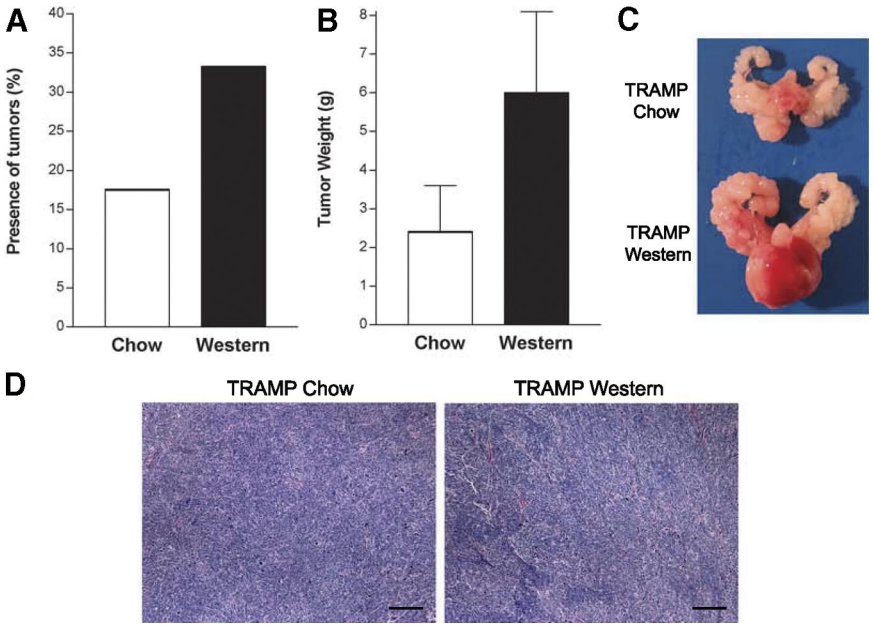

Figure 1. Increased tumor incidence and burden in TRAMP mice fed a Western-type diet. **A:** Twenty-eight-week-old TRAMP male mice fed either a chow or a Western diet were sacrificed and examined for the presence of gross and bulky prostate tumors. Results are expressed as percentage of mice that developed gross prostate tumors that made it impossible for the individual dissection of the different prostatic lobes (n = 17 and 18 for chow and Western group, respectively). **B:** When present, prostate tumors were excised and weighed. Results are represented as the average tumor weight in g of tumor per animal ± SE (n = 3 and 6 for chow and Western diet group, respectively). **C:** Representative images of GU tracts obtained from 28-week-old TRAMP mice fed a chow or a Western diet, demonstrating the presence of prostatic tumoral masses. **D:** Prostate tumors were fixed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Representative histological images are shown for TRAMP males fed a chow (n = 3) or a Western-type diet (n = 6). Scale bars = 200 μm.

fed a Western diet were shown to develop larger tumors with an average of 6.00 ± 2.09 g as compared to only 2.42 ± 1.17 g for mice fed a chow diet (Figure 1B). Figure 1C shows representative pictures of GU tracts corresponding to 28-week-old TRAMP males. Note the increased tumor size observed in the Western diet group. Histological examination of the prostate gross tumors revealed that all tumors were of high-grade and poorly differentiated adenocarcinomas (Figure 1D).

However, in the majority (83 and 67% in the chow and the Western diet groups, respectively) of 28-week-old TRAMP males a bulky tumor was not present. In those cases, the weight of the whole GU tract was taken as an indirect measure of tumor volume, and then individual prostate lobes were excised and histologically examined. As expected, the GU weight in TRAMP mice was significantly greater than in the corresponding nontransgenic littermates in both chow and Western diet fed mice ($P <$ 0.001) (Figure 2B). Interestingly, hyperplasia of the GU apparatus was significantly enhanced (1.77 ± 0.13 g versus 1.21 ± 0.09 g; $P < 0.001$) in mice fed a Western diet as compared to mice fed a chow diet (Figure 2B). Representative pictures of the different GU tracts are shown in Figure 2A.

Taken together, these data indicate that consumption of a typical Western-type diet results in increased tumor incidence and burden, suggesting an important role for dietary fat and cholesterol in prostate tumor formation.

A Western-Type Diet Increases the Extent and the Histological Grade of Prostate Tumors

In addition to following the tumor incidence and burden, we also examined whether dietary fat and cholesterol could affect tumor aggressiveness. Therefore, ventral and anterior prostatic lobes were obtained from each animal, dissected, sectioned, and histologically examined. Representative histological sections are shown for ventral (Figure 3) and anterior (Figure 4) prostates and for each experimental condition (n = 8–10 mice for each group). As expected, ventral (Figure 3A) and anterior (Figure 4A) lobes obtained from nontrangenic males

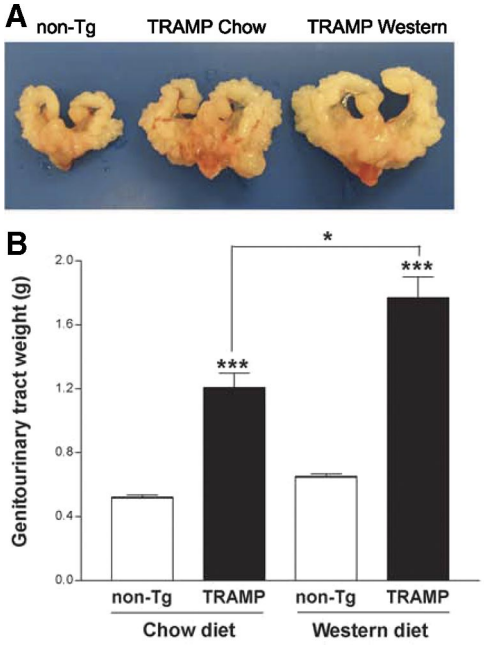

Figure 2. Increased hyperplasia of the GU apparatus obtained from TRAMP mice fed a Western-type diet. **A:** GU tracts from nontransgenic and TRAMP mice when a gross tumor was not present at 28 weeks of age were carefully dissected *en bloc*. Representative pictures of GU apparatus obtained from nontransgenic and TRAMP males fed a chow or Western diet are shown. **B:** After dissection, GU tracts were weighed. This weight was considered to be an indirect measure of the tumor burden in TRAMP mice. Data are expressed in g of GU tract per animal ± SE (n = 14 and 12 for chow and Western diet group, respectively). $^{***}P < 0.001$ nontransgenic versus transgenic littermates; $^{*}P < 0.001$ TRAMP males fed a Western diet versus TRAMP males fed a chow diet.

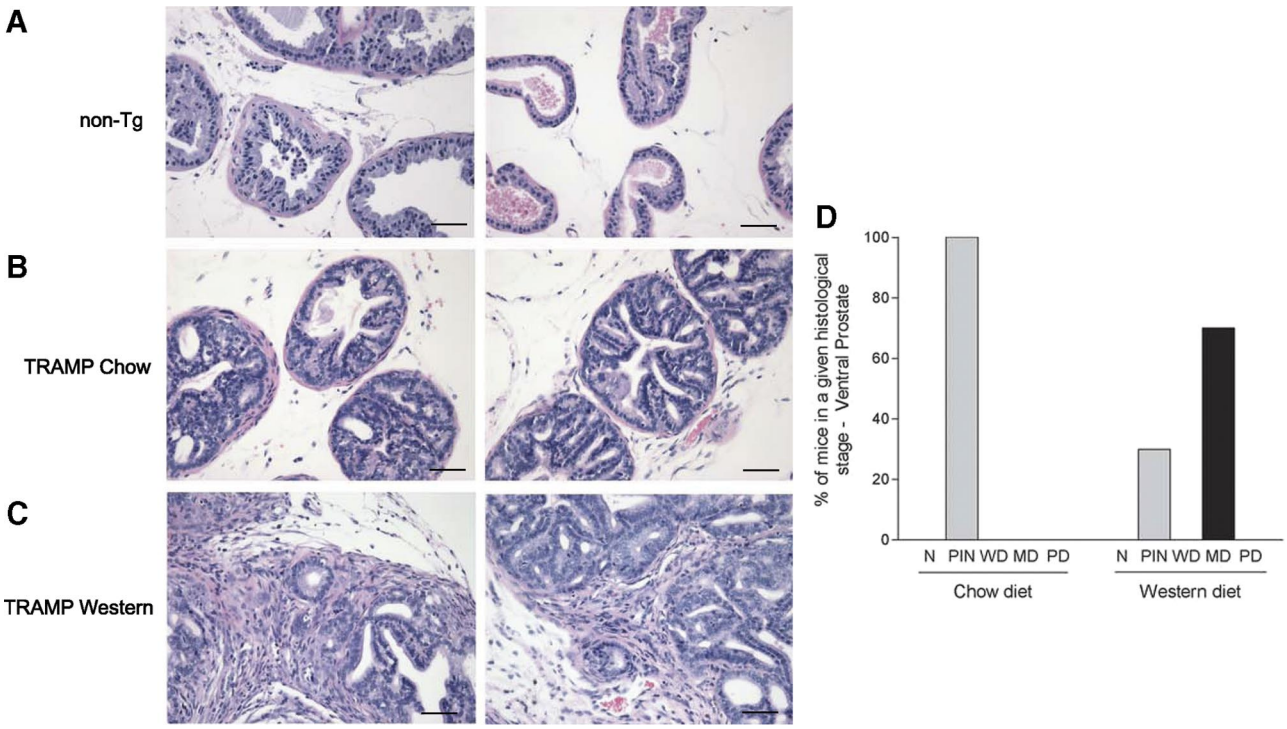

Figure 3. TRAMP mice fed a Western-type diet show advanced ventral prostate carcinogenic lesions. Ventral prostate lobes of nontransgenic and TRAMP male mice fed a chow or a Western diet until 28 weeks of age were microdissected, fixed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Representative histological images are shown of ventral prostates corresponding to nontransgenic males (**A**), TRAMP males fed a chow diet (**B**), and TRAMP males fed a Western diet (**C**). Images were taken at an original magnification of ×40. Each section was graded as normal (N), prostatic intraepithelial neoplasia (PIN), well-differentiated adenocarcinoma (WD), moderately differentiated adenocarcinoma (MD), and poorly differentiated adenocarcinoma (PD) using a scale that had previously been established for TRAMP mice. The percentage of mice in a given histopathological stage is represented (**D**). Eight TRAMP males fed a chow diet and 10 TRAMP males fed a Western diet were examined. Scale bars = 50 μm.

were composed of prostatic glands embedded in a loose stroma. Glands were evenly distributed and regular in shape and size. They were lined by tall columnar cells and showed intraluminal projections with fibrovascular cores. Importantly, no histological differences were observed between ventral and anterior prostates obtained from non-transgenic mice fed a regular chow diet and those obtained from mice fed a Western-type diet (data not shown).

All ventral prostate sections corresponding to TRAMP mice fed a chow diet showed replacement of normal columnar epithelium by hyperchromatic cells forming multilayered groups (Figure 3B). These atypical cells displayed nuclear enlargement, crowding, and loss of polarity as typically seen in high-grade prostatic intraepithelial neoplasia (PIN) (Figure 3D). Most glands maintained regular outline, and there was no change in the surrounding stroma. By contrast, only 30% (3 of 10 mice) of the TRAMP males fed a Western diet were classified as being in the high-grade PIN stage, whereas 70% (7 of 10 mice) of the mice examined showed markedly distended glands and foci of invasion characterized by the presence of irregularly shaped small glands surrounded by desmoplastic stroma (Figure 3C). These prostate tumors were therefore classified as being in the moderately differentiated stage (Figure 3D).

Regarding the anterior prostate analysis, partial replacement of normal columnar epithelium by atypical cells was observed in all of the TRAMP mice fed a chow diet (Figure 4B). By comparison, a higher architectural complexity and a higher replacement by high-grade PIN were observed in TRAMP mice fed a Western diet as compared with mice fed a chow diet (Figure 4C). Moreover, 25% of mice fed a Western diet showed bright eosinophilic secretion and signs of invasion that were not present in any of the mice fed the chow diet (Figure 4D). Therefore, consumption of a Western-type diet results in the worsening of the histological grade of prostate cancer.

Increased Expression of Cell Cycle–Related and Proliferation Markers in the Prostate of TRAMP Mice after Consumption of a Western-Type Diet

Cyclin D1 has been suggested as an appropriate protein marker for prostate tumorigenesis in TRAMP mice because its expression levels have been found to increase gradually concomitant to tumor progression.[32] The expression level of cyclin D1 was therefore examined in the ventral prostates by immunohistochemistry. Confirming the aggravation of the prostate tumors, cyclin D1 expression was shown to be significantly more elevated in tumors obtained from TRAMP mice fed a Western diet compared to samples derived from TRAMP mice fed a chow diet (Figure 5A).

We next examined the expression levels of the proliferating cell nuclear antigen (PCNA), a well characterized

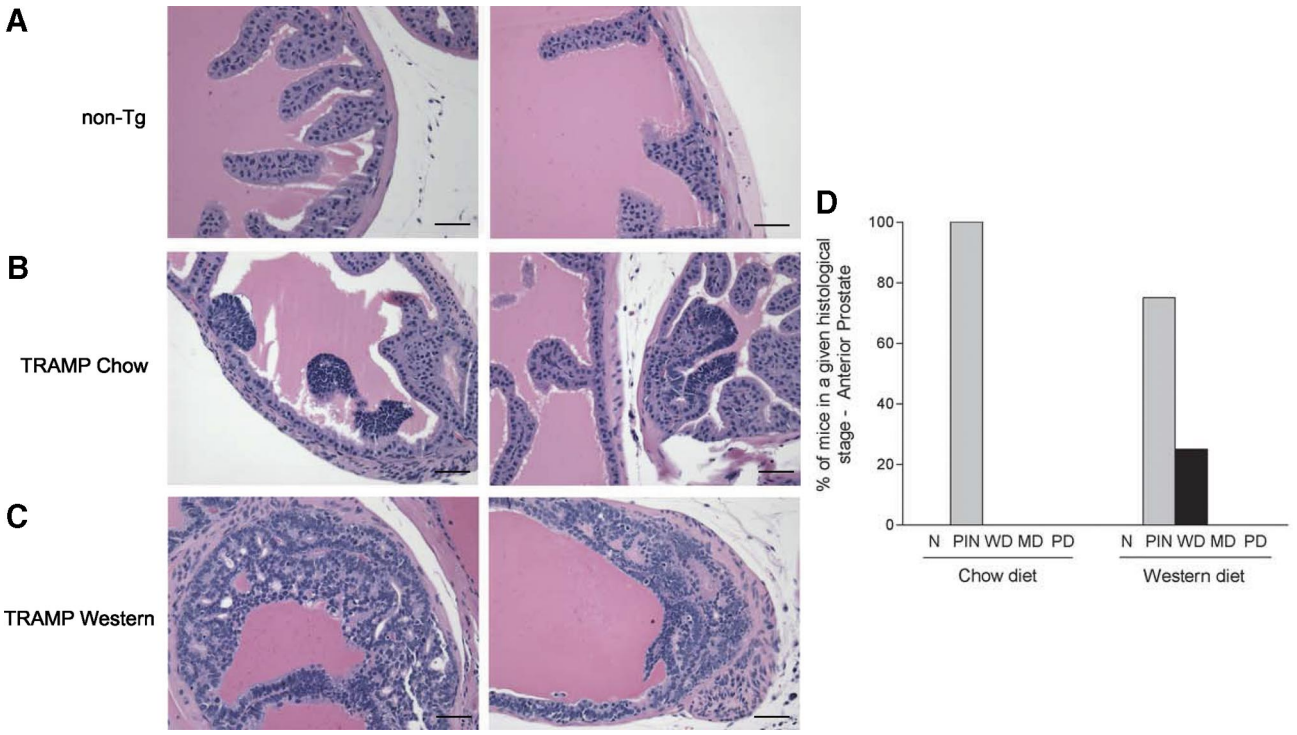

Figure 4. TRAMP mice fed a Western-type diet show more advanced anterior prostate carcinogenic lesions. Anterior prostate lobes of nontransgenic and TRAMP male mice fed a chow or a Western diet until 28 weeks of age were microdissected, fixed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Representative histological images are shown of anterior prostates corresponding to nontransgenic littermates (**A**), TRAMP males fed a chow diet (**B**), and TRAMP males fed a Western diet (**C**). Images were taken at an original magnification of ×40. Each section was graded as normal (N), prostatic intraepithelial neoplasia (PIN), well-differentiated adenocarcinoma (WD), moderately differentiated adenocarcinoma (MD), and poorly differentiated adenocarcinoma (PD) using a scale that had been previously established for TRAMP mice. The percentage of mice in a given histopathological stage is represented (**D**). Nine TRAMP males fed a chow diet and eight TRAMP males fed a Western diet were examined. Scale bars = 50 μm.

marker for cellular proliferation. Immunohistochemical analysis revealed an important increase in the number of PCNA-positive cells in the prostate of TRAMP mice fed a Western diet compared to those of mice fed a chow diet (Figure 5B).

Increased Lung Metastasis in TRAMP Mice Fed a Western-Type Diet

Because the ability of tumor cells to metastasize closely correlates with the pathological grade of the tumor, we also sought to determine whether the consumption of a typical Western-type diet could affect the development of metastases in TRAMP mice. We found that consumption of a Western diet resulted in significantly higher levels of pulmonary metastasis. Thus, 67% (6 of 9 mice) of TRAMP mice fed a Western diet demonstrated the presence of at least one metastatic focus, as compared with 43% (3 of 7 mice) in the group of mice fed a chow diet (Figure 6A). Importantly, control mice lacking the TRAMP transgene did not exhibit any lung metastasis. Furthermore, the number of metastases found in the left lung of each animal was 6.9 times higher in TRAMP mice fed a Western diet, with an average of 3 ± 1.04 metastatic foci versus 0.43 ± 0.2 foci found in the chow diet group (Figure 6B).

Prostate Tumorigenesis Is Associated with Reduced Plasma Cholesterol Levels and Body Fat but only in Mice Fed a Western Diet

Fasting plasma samples were collected from TRAMP males and their nontransgenic littermates at 8, 22, and 28 weeks. Consequently, total plasma cholesterol (Figure 7A) and lipoprotein levels (Figure 7, B and C) were determined before the dietary intervention had started (8 weeks), in the middle of the dietary intervention but as the tumorigenic process had just started (22 weeks), and at the time of sacrifice (28 weeks). As expected, no differences in plasma cholesterol levels were found between TRAMP males and their non-transgenic littermates before the dietary intervention had started (Figure 7A, 8 weeks). Also, consumption of a Western diet resulted in significantly increased plasma cholesterol levels as compared with mice fed a chow diet at both 22 and 28 weeks of age ($P < 0.001$). Interestingly, a significant reduction in plasma cholesterol levels was observed in 28-week-old TRAMP mice fed a Western diet compared to age-matched nontrangenic mice fed the same diet ($P < 0.05$). This suggests that tumor formation was responsible for this reduction. The same effect was not observed in mice fed a chow diet. A more detailed analysis of the type of lipoprotein affected revealed that HDL particles were

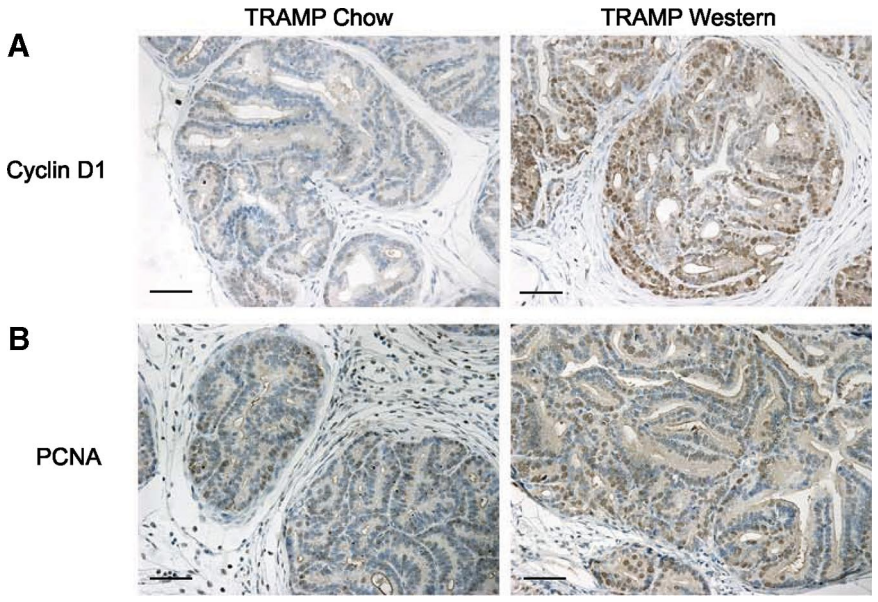

Figure 5. Increased cellular proliferation in the ventral prostate of TRAMP mice fed a Western-type diet. Ventral prostate lobes from 28-week-old TRAMP male mice fed a chow or a Western diet were microdissected, fixed, embedded in paraffin, sectioned at 5 μm, and subjected to immunohistochemical analysis for cyclin D1 (**A**) and PCNA (**B**). Representative images are shown for each dietary condition. Four TRAMP males fed a chow diet and four TRAMP males fed a Western diet were examined. Original magnification, ×40. Scale bars = 50 μm.

reduced in 28-week-old mice developing cancer compared to age-matched nontransgenic mice when they were fed a Western diet (Figure 7C), but not when fed a chow diet (Figure 7B).

Total body weight and epididymal fat weight were determined for each mouse at the time of sacrifice. Consumption of a Western diet resulted in increased body weight and epididymal fat content compared to mice fed a chow diet (Figure 7, D and E, respectively; $P < 0.001$). As demonstrated for plasma cholesterol levels, 28-week-old TRAMP mice showed significant reductions in body (Figure 7D; $P < 0.05$) and epididymal fat (Figure 7E; $P < 0.001$) weight compared to age-matched nontransgenic mice when fed a Western diet, but not when fed a chow diet. These data suggest that the more advanced carcinogenic process observed in TRAMP mice fed a Western diet is responsible for the reductions in plasma cholesterol and body and epididymal fat weight.

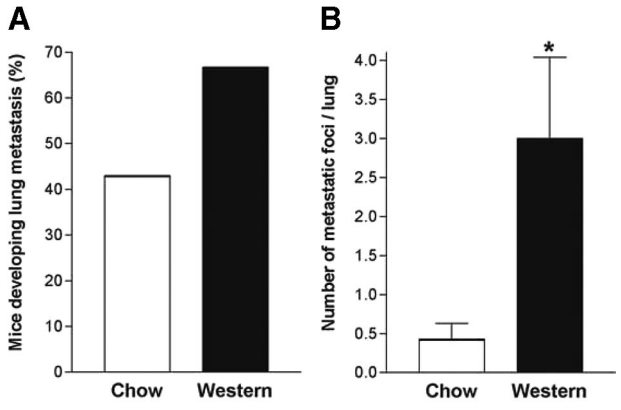

Figure 6. Increased lung metastasis in TRAMP mice fed a Western-type diet. **A:** After removal of the GU tract, 2 ml of 10% buffered formalin were injected through the trachea into the lungs of 28-week-old TRAMP male mice until the lungs were completely inflated and filled with formalin. Lungs were then excised and placed into formalin for 24 hours. The left lung of each animal was paraffin-embedded, sectioned every 50 μm, and stained with hematoxylin and eosin. Lung sections were then examined under a microscope using a ×10 objective for the presence of metastatic foci (defined as a cluster of a minimum of 10 cells). Results are expressed as percentage of mice that developed at least one lung metastatic foci (n = 7 and 9 for chow and Western group, respectively). **B:** The number of metastatic foci was scored for each mouse. Results are represented as the average number of metastatic foci per lung ± SE (n = 7 and 9 for chow and Western diet group, respectively). *$P < 0.05$ Western diet-fed versus chow diet-fed animals.

TRAMP Mice Fed a Western-Type Diet Show Increased Expression of the HDL Receptor SR-BI and Increased Tumor Angiogenesis

Finally, we examined the expression of the protein SR-BI responsible for the selective uptake of cholesteryl ester from HDL. As depicted in Figure 8, TRAMP male mice fed a Western diet showed a striking increase in the expression of SR-BI in the ventral prostate compared to age-matched samples derived from TRAMP mice fed a chow diet. These data suggest that increased levels of SR-BI could account for increased cholesterol uptake by the prostate gland.

Because increased plasma cholesterol levels have previously been correlated with increased angiogenic activity,[33] we also examined microvessel density in tumors obtained from these mice (Figure 9, A and B). For these experiments, tissue sections were stained with CD31 antibody, a specific marker of endothelial cells. Microvascular density was remarkably increased in prostate tumors obtained from animals fed a Western-type diet. These data suggest that increased plasma cholesterol levels are associated with increased angiogenesis in prostates obtained from the TRAMP animal model.

Discussion

Several pathological and epidemiological studies have shown that the prevalence of prostatic intraepithelial neoplasia (PIN) is similar across many populations despite enormous geographic differences in clinically-apparent

prostate cancer incidence and mortality rates. As a consequence, the highest priority should be given to the identification of the factors by which prostate tumor cells progress from occult tumors to more aggressive neoplasms. Lifestyle and dietary factors are certainly reasonable candidates, because both epidemiological and prospective studies have revealed their role in cancer promotion and progression rather than in its initiation. There are numerous studies demonstrating that increased dietary fat intake is associated with elevated prostate cancer risk.[8–11] However, this is still a matter of controversy because more recent studies have failed to confirm these observations and actually suggest no relationship between dietary fat intake and risk of developing prostate cancer.[12,34,35]

Because of the long natural progression of prostate cancer along with the inherent difficulties in performing dietary interventions in human trials, mouse models are commonly used to examine various dietary interventions. Xenograft mouse models of cancer are generally created by a subcutaneous injection of human cancer cell lines into immunodeficient mice. This approach has provided evidences for a reduced growth of tumors established from human prostatic adenocarcinoma cells LNCaP or LAPC-4 in mice fed low-fat diets when compared to mice fed high-fat diets.[36–38] In the present study, TRAMP mice were placed on a Western-type diet. With this diet, prostate cancer progression was assessed and compared to mice fed a chow diet. We show that a Western diet accelerates tumor onset and progression. In particular, consumption of a Western diet resulted in increased hyperplasia of the GU apparatus, as well as increased tumor incidence and size when present in the form of grossly evident prostate tumor masses. Supporting these results, histopathological examination of the individual ventral and anterior prostatic lobes reveals the presence of more advanced carcinogenic lesions in mice exposed to the Western diet. In agreement with our results, others have shown that a high-fat diet had a marked promotional effect on prostate carcinogenesis both in rats[8,39] and in another transgenic mouse model of prostate cancer.[40] In the latter study, mice that are genetically predisposed to developing prostate cancer were placed on a low-fat diet and showed a 27% lower incidence of invasive prostate cancer relative to mice fed a high-fat diet. By contrast, dietary fat did not influence the incidence and histologi-

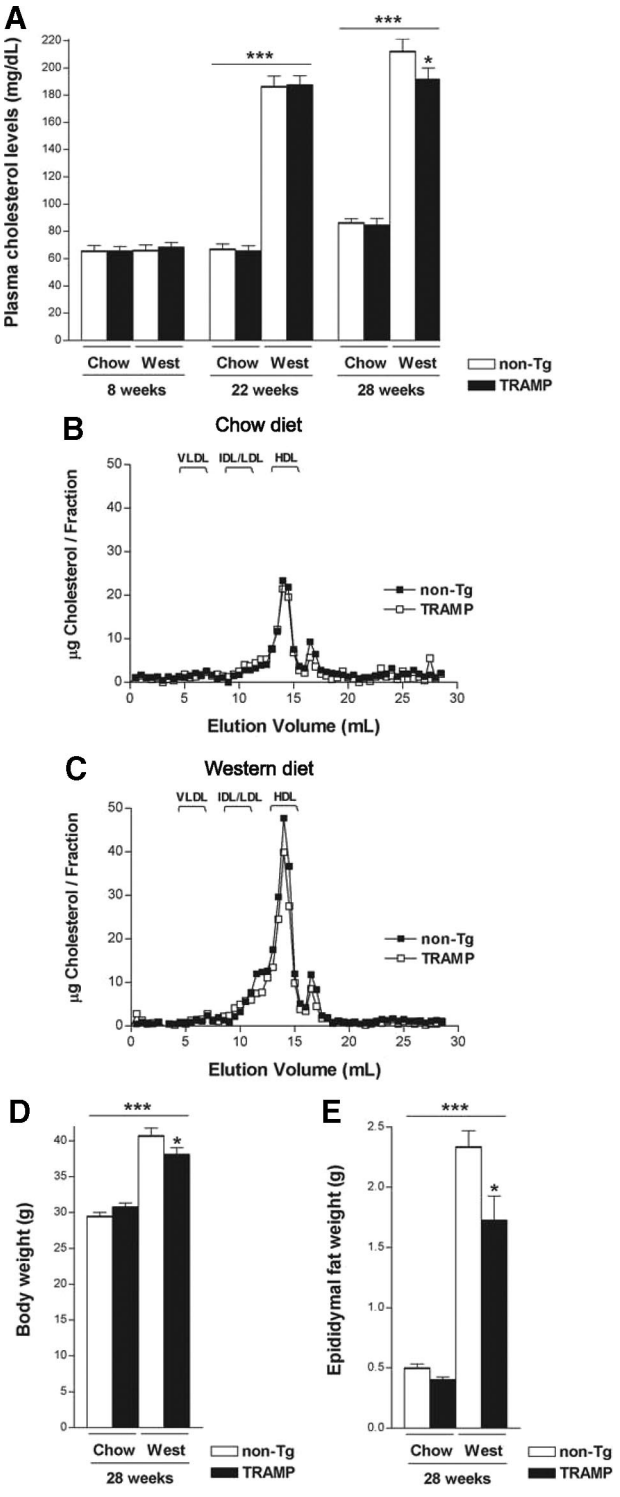

Figure 7. TRAMP mice fed a Western-type diet showed decreased plasma cholesterol levels and body and epididymal fat weights after tumor development. **A:** Total plasma cholesterol content was determined from fasting plasma samples obtained from TRAMP male mice and their non-transgenic littermates using a colorimetric assay. Plasma from 8, 22, and 28 weeks of age mice were analyzed. Total serum cholesterol is expressed in mg of cholesterol/dL ± SE (n = 19 and 17 for nontransgenic and TRAMP mice fed a chow diet, respectively, and n = 17 and 18 for nontransgenic and TRAMP mice fed a Western diet, respectively). $^{***}P < 0.001$ Western diet-fed versus chow diet-fed animals; $^{*}P < 0.05$ TRAMP versus nontransgenic males fed a Western diet. **B** and **C:** Fasting plasma samples isolated from 10 mice on each experimental condition at 28 weeks of age were pooled and loaded atop two Superose 6 columns. Fractions were collected and then analyzed for their cholesterol content using a colorimetric assay. **B:** Lipoprotein profiles obtained from 28-week-old nontransgenic and TRAMP male mice fed a chow diet. **C:** Lipoprotein profiles obtained from 28-week-old nontransgenic and TRAMP male mice fed a Western diet. **D:** At the time of sacrifice, total body weight was determined for each mouse. Data are expressed in g of body weight ± SE (n = 19 and 17 for nontransgenic and TRAMP mice fed a chow diet, respectively, and n = 17 and 18 for nontransgenic and TRAMP mice fed a Western diet, respectively). $^{***}P < 0.001$ Western diet-fed versus chow diet-fed animals; $^{*}P < 0.05$ TRAMP versus nontransgenic males fed a Western diet. **E:** At the time of sacrifice, epididymal fat was dissected and weighed for each mouse. Data are expressed in g of fat ± SE (n = 19 and 17 for nontransgenic and TRAMP mice fed a chow diet, respectively, and n = 17 and 18 for nontransgenic and TRAMP mice fed a Western diet, respectively). $^{***}P < 0.001$ Western diet-fed versus chow diet-fed animals; $^{\#}P < 0.001$ versus TRAMP versus nontransgenic males fed a Western diet.

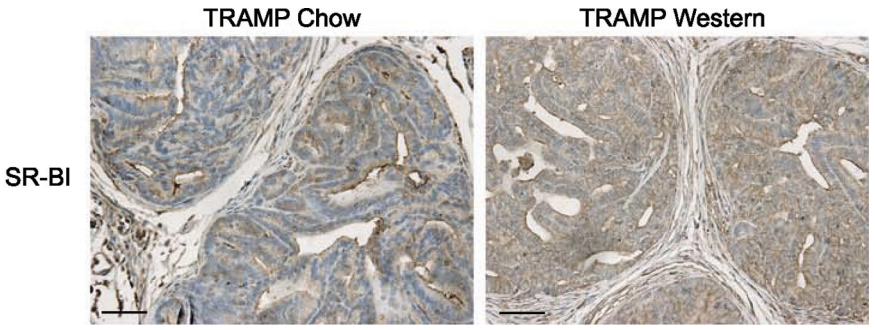

Figure 8. TRAMP mice fed a Western-type diet showed increased expression of SR-BI in the ventral prostate. Ventral prostate lobes from 28-week-old TRAMP male mice fed a chow or a Western diet were microdissected, formalin-fixed, paraffin-embedded, sectioned at 5 μm, and subjected to immunohistochemical analysis for SR-BI. Representative images are shown for each dietary condition. Four TRAMP males fed a chow diet and four TRAMP males fed a Western diet were examined. Original magnification, $\times$40. Scale bars = 50 μm.

cal patterns of prostatic cancer induced by N-nitroso-bis(2-oxopropyl)amine[41] or sex hormones[42] in rats. Therefore, the role for dietary fat remains a matter a controversy and may not be critical during the development of prostate cancer.

The TRAMP model has been extensively validated as a model for the study of prostate carcinoma progression.[28,31,43] Compared to xenograft models, genetically-engineered mouse models used for the study of prostate cancer have many advantages. They more adequately mimic the human disease clinically, genetically, and histopathologically. In genetically-modified mouse models of cancer, normal cells spontaneously evolve to hyperplastic, dysplastic and finally progress to a more malignant stage, a process that cannot be observed in xenograft models because the injected cells are already fully established transformed cells. Moreover, in genetically-modified mouse models of cancer, tumor cells develop in concert with their native environment, as well as with the exposure to the proper growth factors, hormonal, and angiogenic milieu, and in the setting of an intact immune system. Therefore, genetically-engineered models provide more reliable results and are better predictors of the human disease compared to xenograft models. These observations have already been made in the evaluation of the antitumor activity of thiazolidinediones or farnesyltransferase inhibitors.[44,45] Accordingly, our experiments were performed using the TRAMP mouse model. As a result, our studies clarify and validate the role of a Western-type diet previously examined in xenograft models during the development of prostate cancer.

The specific components of the Western-type diet that may predispose to prostate cancer are a matter of controversy. A role for cholesterol, which is a prominent component of this typical diet, in prostate cancer incidence and progression has recently been suggested through a number of epidemiological and preclinical studies.[20,33,46–49] In the present study, a Western diet was used to elevate the levels of circulating plasma cholesterol in TRAMP mice. As a consequence of elevated plasma cholesterol levels, tumor onset and progression were accelerated. In our study, we have used a typical Western diet, which is enriched in both fat and cholesterol. Importantly, very few studies have been designed to assess the unique role of cholesterol in cancer growth and progression. Preclinical data indicates that treatment of patients with statins, which are the most common cholesterol-lowering drugs, can play a chemopreventative role against the development of various human cancers by inhibiting cellular proliferation. In previous studies, it was shown that statins could induce apoptosis and inhibit angiogenesis or neovascularization.[50,51] Nevertheless, the effect of statin treatment on cancer remains controversial.[51] In addition, this effect may not only be

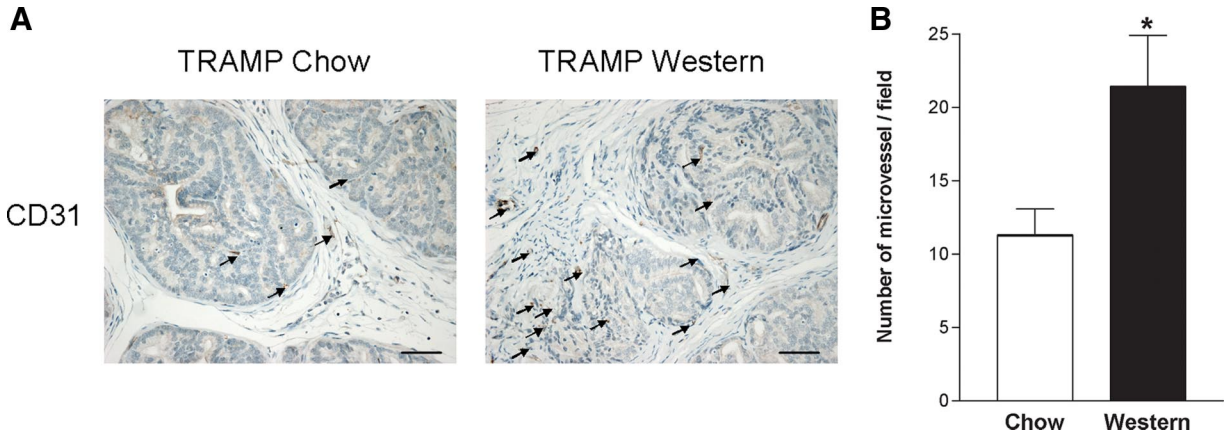

Figure 9. Increased plasma cholesterol is associated with increased tumor angiogenesis in TRAMP mice. Ventral prostate lobes from 28-week-old TRAMP male mice fed a chow or a Western diet were microdissected, formalin-fixed, paraffin-embedded, sectioned at 5 μm, and subjected to immunohistochemical analysis for CD31. **A:** Representative images are shown for each dietary condition. Three TRAMP males fed a chow diet and three TRAMP males fed a Western diet were examined. Original magnification, $\times$40. Scale bars = 50 μm. **Arrows** indicate the presence of CD31-positive blood vessels. **B:** Microvessel quantification was performed by counting the number of CD31-positive vessels in three representative fields for each section. Results are expressed as average $\pm$ SE. Significant difference $^*P < 0.05$.

due to their hypocholesterolemic action but could also be attributed to the so-called pleiotropic effect of statins.[16] Supporting a direct role for plasma cholesterol levels in tumor progression, another hypocholesterolemic strategy has been evaluated. The ezetimibe drug has recently been shown to reduce tumor growth after the injection of prostate cancer LNCaP cells in SCID mice.[33] Moreover, studies from Zhuang et al have shown that raising serum cholesterol by consumption of a cholesterol-rich diet resulted in accelerated tumor formation in SCID mice harboring LNCaP cell-derived prostate xenograft tumors.[48] Importantly, elevated plasma cholesterol levels have been shown to increase membrane cholesterol content, which leads to an expansion of the lipid raft compartment. As a consequence, this physical change has been shown to affect several oncogenic signaling pathways that increase Akt phosphorylation and reduce apoptosis in the xenografts tumors. Finally, an interesting study performed by Kimura et al has focused on assessing the individual effects of consumption of a high-fat or a high-cholesterol diet on tumor growth after the subcutaneous implantation of Lewis lung carcinoma cells.[49] Both diets increase plasma total cholesterol levels, but increased body and epididymal fat weight have only been observed in mice fed the high-fat diet. Interestingly, both the high-fat and the high-cholesterol diets show accelerated tumor growth and metastasis, which suggest that the hypercholesterolemic effect caused by the two diets is sufficient to promote tumor growth by itself.

The scavenger receptor class B type I (SR-BI) is a plasma membrane receptor responsible for the selective uptake of HDL-cholesteryl ester by cells.[52] Previous works have shown that, in transformed mammary epithelial cells, SR-BI expression regulates cholesterol uptake and cellular proliferation.[53,54] Importantly, others have shown that SR-BI is actually expressed in the prostate.[55] In the present work, we show that SR-BI is not only expressed in transformed prostatic epithelial cells, but its expression is increased in prostate tumors obtained from mice fed a Western diet. Therefore, SR-BI may be responsible for an increased cholesterol uptake by the tumor and may indirectly regulate tumor development. This uptake may allow the maintenance of tumor progression by replenishing cellular cholesterol stocks. Previous studies have shown that SR-BI protein levels are inversely correlated with plasma HDL-cholesterol levels. For example, fibrate treatments of mice have been shown to decrease hepatic SR-BI protein levels and also increase plasma HDL-cholesterol levels.[56] On the other hand, macrophage SR-BI expression has been shown to be associated with altered plasma HDL-cholesterol levels in a human population.[57] Therefore, our studies suggest that SR-BI expression in the tumorigenic prostate may be responsible for the decreased in plasma HDL-cholesterol levels.

In addition, we have also observed an increased angiogenic response in tumors obtained from mice fed a Western-type diet compared with those obtained from mice fed a chow diet. Plasma HDL-cholesterol levels have been shown to play a role in the regulation of angiogenesis.[58] Interestingly, studies by Li et al have indicated that HDL binding to SR-BI may induce eNOS activation, which is responsible for enhanced angiogenesis via the production of nitric oxide.[59] Taken together, our data suggest that increased plasma cholesterol is associated with increased tumor cholesterol uptake and proliferation. In addition, this enhanced tumorigenesis may be sustained by the increased angiogenesis observed in animals fed a Western-type diet.

Very few studies have specifically addressed the role of dietary fat and cholesterol on distant site metastasis formation. In the present study, our data indicate that the incidence of pulmonary metastasis in TRAMP mice was 24% higher by feeding a Western diet compared to the incidence observed in control animals fed a chow diet. Also, in the typical Western-type diet group, the number of metastatic foci present in the lung was found to be greater. These results are in agreement with a previous study from Kimura et al who found a 38% increase in metastasis incidence after the subcutaneous implantation of highly metastatic lung carcinoma cells to male C57Bl/6J mice fed a high-fat or high-cholesterol diet compared to control animals.[49] Moreover, in another study in which the MDA-MB-435 human breast cancer cell line was injected, a 38% incidence of macroscopic lung metastasis was detected in mice fed a low-fat diet whereas this percentage was found to be 67% in the high-fat diet group.[60] The mechanisms by which dietary fat or cholesterol could be promoting metastasis formation need to be further evaluated. Nonetheless, we can propose that cholesterol increases the aggressiveness of the tumor, and, by extension, this may lead to increased metastasis formation in animals fed a Western-type diet.

In summary, our data show that increased plasma cholesterol levels and fat deposits caused by the consumption of a typical Western diet accelerates prostate tumor progression and exacerbates its aggressiveness in a mouse model of prostate cancer. These data suggest that the increased availability of cholesterol and fat may be responsible for the more advanced carcinogenic process observed in the TRAMP mice fed a Western diet. Our data provide new evidences for a role of these dietary components on tumor progression. Although more physiologically relevant than the xenograft models, important biological differences still exist between murine and human malignancies. Therefore, whether these benefits in animal studies translate into benefits in humans will need to be further elucidated. Nevertheless, given the overall health benefits of controlling plasma cholesterol levels and body weight, in addition to the possible benefits offered to the prostate, it appears that managing cholesterol metabolism would be a prudent plan for all men.

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